REMARKS

This application is a division of United States Application No. 09/545,139 filed April 7, 2000, which is a division of United States Application No. 08/950,673, now patent No. 6,071,948, which is a continuation of United States Application No. 08/468,792, now patent No. 5,712,291, which is a continuation of United States Application No. 08/168,817, now patent No. 5,629,327; which is a continuation in part United States Application No. 08/950,673, now patent No. 5,071,948, all of which relate to methods of treating diseases associated with angiogenesis using thalidomide.

Interview Summary

Applicant thanks Examiner Cook for the courtesy she extended to Agent for Applicant, Nicholas J. DiCeglie, Jr., during a telephone interview held on October 15, 2003. During the interview, Examiner Cook upheld the rejection under 35 § U.S.C. 103(a), asserting that because no one compound is known to be effective against all cancers, a compound can be effective to treat some cancers while being inoperative against others. Applicant respectfully disagrees for the reasons below.

The Claimed Invention

Claims 23, 25-31 and 32-58 are pending in this application. Claims 24 and 32 have been cancelled. Claims 23, 25, 34, 41, 42 and 49-51 have been amended as shown above. No new matter has been introduced by the present amendments, and their entry is respectfully requested. By the amendments, Applicant does not acquiesce to the propriety of any of the Examiner's rejections and does not disclaim any subject matter to which Applicant is entitled. *Cf. Warner Jenkinson Co. v. Hilton-Davis Chem. Co.*, 41 U.S.P.Q.2d 1865 (U.S. 1997). Further, Applicant reserves the right to prosecute the subject matter of any canceled or amended claim in one or more continuation, continuation-in-part, or divisional applications.

The claims encompass methods of inhibiting tumor formation and metastasis of tumors in humans using an angiogenesis-inhibiting amount thalidomide. In certain embodiments (claims 27-29, 36-38 and 44-46), the angiogenesis-inhibiting amount of thalidomide is about 300 mg/kg/day or less.

Rejection Under 35 U.S.C. § 103(a)

Claims 23-58 stand rejected under 35 U.S.C. § 103(a) over Sugiura, K. and H.M. Wuest "Effect of Thalidomide on Transplantable Mouse, Rat, and Hamster Tumors" *GANN*, 55, 57-60, February 1964 ("Sugiura") or over United States Patent No. 5,399,363 to Liversidge et. al. ("Liversidge"). The Examiner maintains, *inter alia*, that the mere allegation in Sugiura that repeated interperitoneal injections of thalidomate (1000 mg/kg/day) had a *moderately inhibitory* effect on only one tumor model — namely, lewis bladder carcinoma — is sufficient to render the claims obvious despite the fact that ***twenty-four*** other tumors were reported to be non-responsive to thalidomide. Applicant respectfully traverses the rejection.

It is well settled that a prior art reference must be considered in its entirety when relied upon by a rejection under 35 U.S.C. § 103. *Ashland Oil, Inc. v. Delta Resins & Refractories, Inc.* 277 U.S.P.Q. 657 (Fed. Cir. 1985). Moreover, "in determining whether ... a suggestion can be fairly gleaned form the prior art, the full field of the invention must be considered." *In re Dow Chemical Co.* 5 U.S.P.Q.2d at 1531 (Fed. Cir. 1988). One of ordinary skill in the art is charged with the knowledge of all the relevant literature. *Id.* at 1532. Thus, it is improper to view the disclosure of a prior art reference separate from the teachings of others. The determination of obviousness cannot be performed in a vacuum but must be viewed with the understanding of those the art as a whole. *Cf.* at 1532.

The claims pending in this application are directed to methods of inhibiting tumor formation and inhibiting tumor metastasis in humans using an angiogenesis-inhibiting amount thalidomide. Contrary to the Examiner's contention, Sugiura teaches away from the method of inhibiting tumor formation as claimed by the Applicant. For example, Sugiura states "thalidomide had ***no inhibitory effect*** on the growth of 18 kinds of mouse, rat, and hamster tumor." Sugiura, p. 59 (emphasis added). Further, Sugiura allegedly teaches that thalidomide only had *slight inhibiting effect* on six of the tumors treated and only effected *moderate inhibition* of one tumor at 1000 mg/kg/day. Sugiura clearly would not have provided those of ordinary skill in the art with any expectation of successfully obtaining the claimed invention. *In re Fine,* 837 F.2d 1071, 1075, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988). Thus, applicant respectfully requests that the rejection be withdrawn.

Applicant's interpretation of Sugiura is consistent with the understanding of those of ordinary skill in the art prior to this invention. As evidence of this, Applicant submits a copy of H. Mükter, "Thalidomide and Tumor" *Antimicrobial Agents and Chemotherapy* 1965, 531-538, ("Mükter"), enclosed herewith as Exhibit A. As the Examiner will see, Mükter describes Sugiura as showing ***no effect*** on twenty-four tumors of

mouse, rat and hamster, and as only having a ***weak*** effect for Lewis Bladder-carcinoma. Clearly, those skilled in the art recognized that Sugiura demonstrated that thalidomide was ***not active*** against cancer. Thus, this reference cannot support the Examiner's obviousness rejection in light of the teachings of the art as a whole.

Significantly, human studies conducted prior to Applicant's invention, which are more relevant to the pending claims, also taught away from Applicant's invention. For example, the Examiner's attention is directed to Grabstald and Golbey "Clinical experiences with thalidomide in patients with cancer," *Clinical Pharmacology and Therapeutics*, 6, 298-302, 1965 ("Grabstald") enclosed herewith as Exhibit B.[1] Grabstald observed the effects of thalidomide in 71 patients with a variety of cancers. Grabstald concludes that "in the absence of more definite evidence of pharmacologic or anticancer effects in man, *we conclude that further random trials of this drug against cancer in man* ***are not indicated***." Grabstald, at page 301 (emphasis added). In sum, one of ordinary skill in the art, in view of Mükter and Grabstald, would not have taken from Sugiura any expectation of successfully practicing the claimed invention.

Applicant further submits that Examiner's contention that no one compound is known to be effective against all cancers does not rectify this inadequacy and is, indeed, irrelevant to the Examiner's contention that the present claims are obvious.[2]

Liversidge does not remedy the deficiencies of Sugiura. The Examiner relies on the statement in Liversidge that "the anticancer agent can be an immunosuppressive drug, such as, for example, cyclosporine, azathioprine, sulfasalazine, methoxsalen, and thalidomide" to render Applicant's method of inhibiting tumor formation using an angiogenesis-inhibiting amount thalidomide obvious. Liversidge, column 3, lines 4-49. Applicant maintains that this statement does not suggest all of the claim limitations, nor does it satisfy the legal requirement for obviousness.

At the time of Liversidge, thalidomide was not considered useful for the treatment of cancer. Liversidge's misleading statement does not change that fact. At the

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[1] Applicant respectfully requests that the Examiner execute the enclosed PTO Form 1449 to demonstrate that Exhibit A and Exhibit B were considered.

[2] Examiner appears to be arguing that Sugiura may invite the reader to experiment with thalidomide, despite the strong teaching away by Mükter and Grabstald, *i.e.*, that Sugiura arguably renders the claimed invention obvious to try. However it is well established that "obvious to try" cannot form a basis for a rejection under § 103. *In re O'Farrel* 853 F.2d 894, 57 USLW 2147, 7 U.S.P.Q.2d 1673 (Fed. Cir. 1988).

relevant time, all of the compounds referred to by Liversidge were known only as immunosuppressive agents, although, thalidomide was therapeutically classified as an immunomodulatory compound and as a sedative. *The Merck Index*, 12th ed., Whitehouse Station: Merck, 1996. Neither of these therapeutic uses suggest that thalidomide can be used to treat cancer. Moreover, Liversidge does not demonstrate that any of the specific immunosuppressive compounds it mentions are effective as anticancer agents. Consequently, its allegation that an "anticancer agent can be an immunosuppressive drug" does not provide an adequate suggestion of Applicant's method of inhibiting tumor formation using an angiogenesis-inhibiting amount thalidomide, much less provide the requisite suggestion of the dosages or specific tumors recited by the pending claims.

Further, Liversidge must be read in the context of what was known by those of ordinary skill in the art prior to the invention. It must be read in conjunction with Sugiura, Mükter, Grabstald and other art at the time. *See, In re Ochiai*, 71 F.3d 1565, 1569, 37 USPQ2d 1127, 1131 (Fed. Cir. 1995). As discussed above, Sugiura, Mükter and Grabstald each teach away from Applicant's invention. Liversidge does not provide any evidence to the contrary. Thus, Liversidge alone, or in combination with Sugiura, does not render the claimed invention obvious.

Finally, the single statement of Liversidge cited by the Examiner would not have provided those skilled in the art with a reasonable expectation of success. *See In re Fine,* 837 F.2d at 1075. Even if one were to choose an immunosuppressive drug, such as thalidomide, from the list of drugs provided by Liversidge, there is no teaching or suggestion that thalidomide would inhibit tumor growth in a human. At most, Liversidge suggests that immunosuppressive agents can be incorporated into the delivery method that Liversidge discloses. Even assuming, *arguendo*, that Liversidge's statement would not have been immediately discounted by those skilled in the art, the statement provides, at most, a mere invitation to experiment. As the Examiner is aware, an allegation that something may have been "obvious to try" cannot form an adequate basis for a rejection under § 103. *In re O'Farrel* 853 F.2d 894, 57 USLW 2147, 7 U.S.P.Q.2d 1673 (Fed. Cir. 1988).

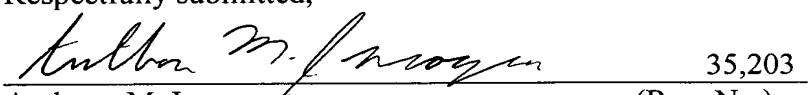
In sum, one of ordinary skill in the art would immediately recognize upon reading Liversidge that immunosuppressive agents are not antitumor agents, and would have had no expectation of successfully practicing the claimed inventions. Applicant therefore respectfully submits that claims 23-57 are not obvious over Suguira or Liversidge, and requests that the rejection of claims 23-57 under 35 U.S.C. §103(a) be withdrawn.

Conclusion

Applicant respectfully requests that the above remarks and accompanying documents be entered in the file of this application. Applicant also respectfully requests withdrawal of the outstanding rejections. No fee is believed due. However, please charge any required fees to Pennie & Edmonds LLP Account No. 16-1150.

Respectfully submitted,

Date: December 3, 2003



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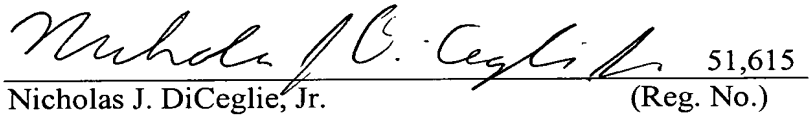
Anthony M. Insogna (Reg. No.)

PENNIE & EDMONDS LLP
12750 High Bluff Drive, Suite 300
San Diego, California 92130
(858) 314-1200

By: 

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Nicholas J. DiCeglie, Jr. (Reg. No.)

PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosures

Antimicrobial Agents and Chemotherapy–1965

Thalidomide and Tumor

H. MÜCKTER

Research Laboratories, Chemie Grunenthal GmbH, Stolberg/Rheinland, Germany

Abstract

Autonomously growing transplanted tumors, with some exceptions, are not influenced by thalidomide. Hormone-dependent tumors induced by 7,12-dimethylbenz[a]anthracene (DMBA) in Sprague-Dawley rats respond to prophylactic application of thalidomide as well as to curative treatment with this substance. Under prophylactic application, thalidomide delays the appearance and growth of DMBA-induced tumors. When given therapeutically, thalidomide influences the manifestation and, to a certain extent, the growth of DMBA-induced tumors. The effect is limited by the size of the tumors at the time of first application, and by the duration of treatment. The spontaneously developing, virus-induced, and hormone-independent mammary-cancers of C_3H/O_{20}-mice are only temporarily influenced by thalidomide. The mechanism of antitumor action of thalidomide differs fundamentally from the cytostatic action of cyclophosphamide, in that it seems to exert its effect mainly or perhaps exclusively through the endocrine system.

During experimental evaluation of thalidomide in 1954-1955, we found that this compound does not influence the Ehrlich carcinoma of albino mice.

After McBride (1961) and Lenz (1961) reported that thalidomide is teratogenic in man, comprehensive investigations on numerous transplanted tumors were conducted in various laboratories. In nearly all of these experiments, no indication of cytostatic action of thalidomide was observed (Table 1).

Recent biochemical observations and considerations led us to study the effect of thalidomide both on chemically induced and spontaneously developing tumors. The main results of these experiments conducted over several years in the Grunenthal laboratories form the subject of this report.

Table 1. *Effect of thalidomide on transplanted tumors*

Year	Investigators	Type of tumor	Effect*
1956	Kunz, Keller, and Mueckter	Ehrlich-carcinoma of the mouse	ϕ
1963	DiPaolo	10 different types	8 ϕ 2 ±
1963	Juret and Aubert	Ehrlich-carcinoma of the mouse Epithelioma of the rat	ϕ
1963	Bach, Bichel, and Hojgaard	Leukemia and carcinoma of the mouse	ϕ
1963	Pagnini and DiCarlo	Ehrlich-carcinoma of the mouse Myeloma Oberling-Guerin of the rat	ϕ
1964	Sugiura and Wuest	24 tumors of mouse, rat, and hamster	ϕ
		Lewis bladder-carcinoma	±

*Symbols: ϕ = no effect; ± = weak effect.

Chemically Induced Tumors

Prophylaxis experiments. Following the modus operandi of Huggins, Grand, and Brillantes (1961), female Sprague-Dawley (SD) rats of American origin, between 50 and 65 days old with a mean weight of 160 g, were given a single dose of 20 mg 7,12-dimethylbenz[a]anthracene (DMBA) in 1 ml of sesame oil through a stomach tube. The test substances were mixed with the food (Sniff, Mssrs. Plange, Soest) so as to maintain as far as possible constant blood and tissue levels at the desired concentrations. The food was given to the animals in compressed tablet form. Thalidomide was mixed with the food in a series of 10-fold concentrations starting at 0.001% and rising to 1%. Groups of 15 to 20 rats were fed with pellets containing the required amounts of thalidomide from 2 days before giving DMBA to the end of the experiment. In general, the rats consumed some 15 to 20 g daily, which corresponded according to the various concentrations in the food to a daily intake of from 1 mg (0.001%) to 1,000 mg (1.0%) per kilogram of body weight. In parallel experiments with mice, the daily dose of the substance was about doubled, since the mouse eats about twice as much food per kilogram of body weight as does the rat. During the experiment, the animals were kept in Makrolon cages, and received no additional feeding, but water was supplied ad libitum. The animals were weighed at the start, and at fixed times during the trial.

The great majority of the tumors induced by DMBA occurred in the region of the nipples as mammary new growths. They were evaluated by ascertaining the number and surface area of the tumors at predetermined times. In estimating the tumor areas, we used a modified batch technique by measuring in each case the largest and smallest diameters of the tumors ascertainable percutaneously, and reckoning the product of the two values as the area. We were of course well aware that this could only be an approximation; however, it was adequate for the purposes of the experiment. Besides the number and size of the tumors, we also determined the average latent period, i.e., the time taken for 50% of the tumors to become palpable in each of the treated animals. We also carried out extensive endocrinological and histopathological investigations, the results of which will be given in another publication. In the matter of the duration of the experiment, we followed Huggins (1965) in general, with experiments lasting 180 days from the date of giving the DMBA.

A total of three prophylaxis experiments were carried out by the above methods with a total of 290 animals, 58 in each group. Figure 1 shows the result of one of these three tests for the 1% concentration of thalidomide in comparison with the corresponding control. After 180 days, 5 of 18 treated control rats had died intercurrently. With the exception of 1 animal, all of the remaining 12 showed one or more tumors, a result consistent with those of Huggins (1965). Of the 18 rats thalidomide, there wer deaths, 3 animals deve and the remaining 13 tumors.

The mean latent per tion of the tumors incre with rising thalidomid compared with the co the 1% thalidomide gi reached within the ob of 180 days.

Figure 2 shows t area of the tumors in square millimeters, pl thalidomide concentrat curve). Whereas the control DMBA rats sh area of 1,178.6 mm², t were reduced with ri concentrations, until at average area per anim

Table 2 shows in co thalidomide, and under mental conditions, the fluence of cyclophospl and the estrogen combi (0.001%) plus chlorm which after 10 days wa tenth of the original cause of its toxicity.

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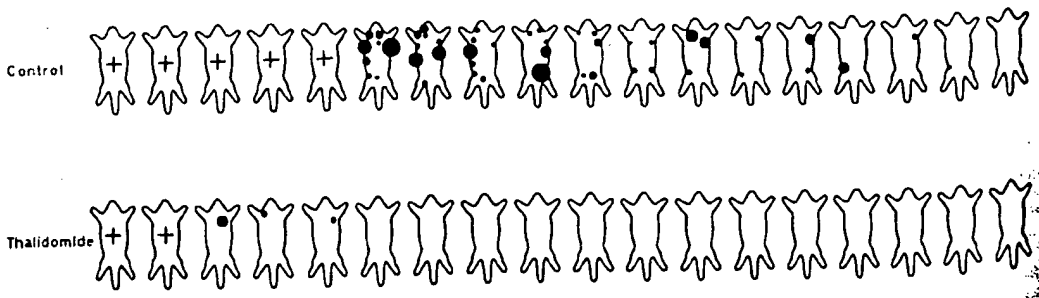


Fig. 1. *Prophylaxis with 1% thalidomide of rats with DMBA-induced tumors.*

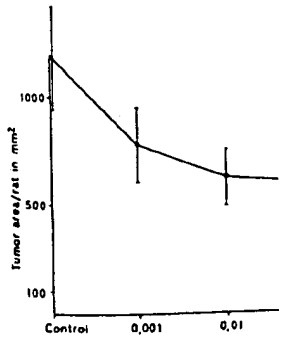


Fig. 2. *Total surface area of thalidomide-treated rats.*

(1965). Of the 18 rats treated with 1% thalidomide, there were 2 intercurrent deaths, 3 animals developed one tumor, and the remaining 13 were free from tumors.

The mean latent period of manifestation of the tumors increased, in general, with rising thalidomide concentrations compared with the controls, and with the 1% thalidomide group it was not reached within the observation period of 180 days.

Figure 2 shows the total surface area of the tumors in each animal in square millimeters, plotted against the thalidomide concentration (dose-effect curve). Whereas the tumors of the control DMBA rats showed an average area of 1,178.6 mm², the surface areas were reduced with rising thalidomide concentrations, until at the 1% level the average area per animal was 63.7 mm².

Table 2 shows in comparison with 1% thalidomide, and under the same experimental conditions, the prophylactic influence of cyclophosphamide (0.0025%) and the estrogen combination, mestranol (0.001%) plus chlormadinone (0.001%), which after 10 days was reduced to one-tenth of the original concentration because of its toxicity.

Although the hormone treatment influenced the manifestation and size of the new growths in the same manner as did thalidomide, the results in the cyclophosphamide-treated rats were the same as in the controls. It is noteworthy that, in addition to high mortality at the start of the experiment, the hormone-treated animals showed a marked inhibition of the growth curve.

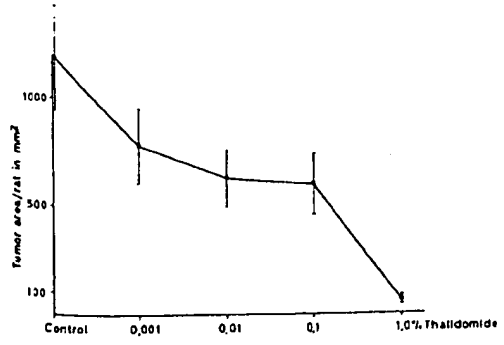


Fig. 2. *Total surface area of DMBA-induced tumors in thalidomide-treated rats.*

To summarize, the prophylaxis experiment showed that, under the conditions of the experiment here described, thalidomide retarded in a most impressive fashion the manifestation and growth of tumors induced in rats by the carcinogen DMBA, and the effect bore a direct relationship to the dose administered. Thalidomide appeared to behave in a manner similar to the sex hormones, and in our experiments cyclophosphamide was found to be devoid of effect.

Curative experiment. SD rats with developed tumors induced by DMBA were divided into groups of 10 animals such that, as far as possible, the average tumor area of each animal in the different groups was about the same. The substances to be tested were incorporated in the food as was done in the prophylaxis experiment. The size and numbers of tumors were recorded, along with the weight, in some experiments after 4 weeks and in others after 5, 10, and 12 weeks. Figure 3 shows the comparison between rats with DMBA tumors after 5 weeks of treatment with 1% thalidomide and untreated animals with tumors. At the start of the trial, the mean tumor area in both groups was about 200 mm².

Whereas the tumors of the controls increased on the average from about 200 to 1,144 mm² during the period of the experiment, the mean tumor surface of the animals given thalidomide decreased from about 200 mm² to about 160 mm² in the same time.

Figure 4 shows the comparison of DMBA tumors under treatment with 1% thalidomide for 4 weeks with the untreated controls. The mean tumor area was about 800 to 1,000 mm² per animal at the start of the experiment. The new growths in the controls increased from 776 to 1,501 mm² per rat, and the mean tumor area in the treated animals increased from 977 to 1,331 mm².

Table 2. *Comparative effect on DMBA-induced tumors in the rat of thalidomide, cyclophosphamide, and the estrogen combination mestranol-chlormadinone*

Drug	Concn in food (%)	Tumors/rat	Tumor area per rat (mm^2)	Increase of body weight (g/rat)
Control	0	5.0	1,421	167
Thalidomide	1	0.56	76	136
Mestranol	0.01*	0.84	126	29
Chlormadinone	0.001*			
Cyclophosphamide	0.0025	4.5	1,563	173

*The concentration of the two hormones was reduced after 10 days to one-tenth of the original concentration because of toxicity.

Figure 5 shows the changes in the DMBA tumor area when 1% thalidomide was given for 4 weeks, and the tumor sizes at the start of the test were 200, 500, and 1,000 mm^2. For clarity, the relative curves for the untreated controls are omitted. This figure shows that, in general, the mean tumor size was reduced to a limited degree by thalidomide treatment if the tumor was relatively small at the beginning of the experiment. The bigger the mean tumor area, that is the older it was at the start of treatment, the weaker the thalidomide action was. This general conclusion does not exclude the fact that individual tumors deviated from this pattern. Thus, occasionally even small tumors did not respond to thalidomide and large growths at times were arrested, or indeed even regressed under thalidomide treatment. No reliable explanation has been found so far for these individual cases.

The number of new tumors which appeared during the treatment in 10 curative experiments is shown graphically in Fig. 6. Whereas in the controls an average of 1.7 new tumors per rat were observed, the corresponding rate of appearance in the thalidomide-treated animals was 0.18. The difference is highly significant ($P < 0.001$).

When the course of treatment was prolonged from 4 to 5 weeks to 8 to 12 weeks it appeared that the number of tumors decreased rather than increased. Because of the small number

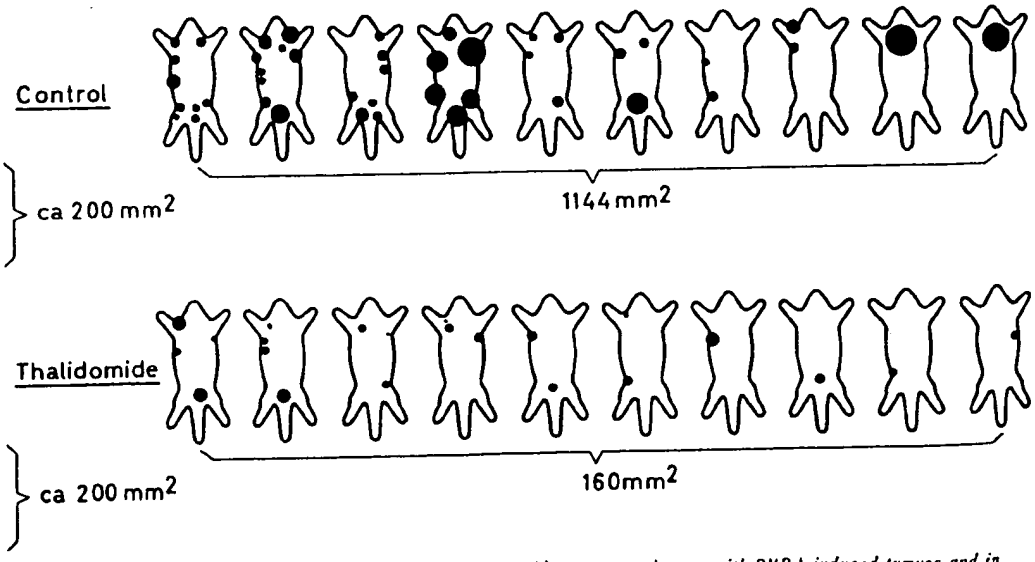


Fig. 3. *Comparison of results after 5 weeks of 1% thalidomide treatment in rats with DMBA-induced tumors and in untreated animals with tumors.*

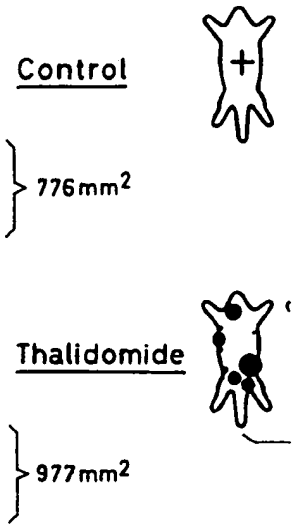


Fig. 4. *Comparison of increas 4 weeks.*

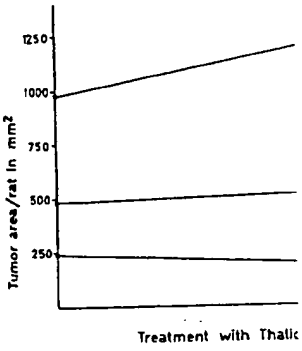


Fig. 5. *Change in size after 1% of tumors having areas of 200, 500 treatment.*

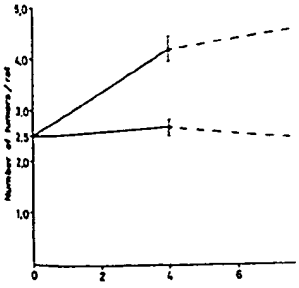


Fig. 6. *Appearance of new tumo 10 curative experiments.*

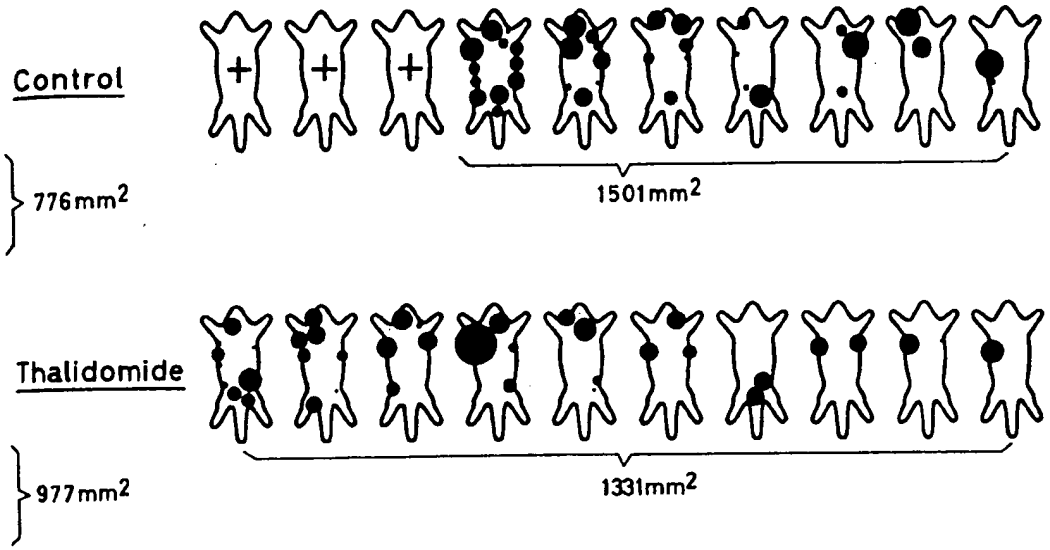


Fig. 4. *Comparison of increase in area of DMBA-induced tumors with and without 1% thalidomide treatment after 4 weeks.*

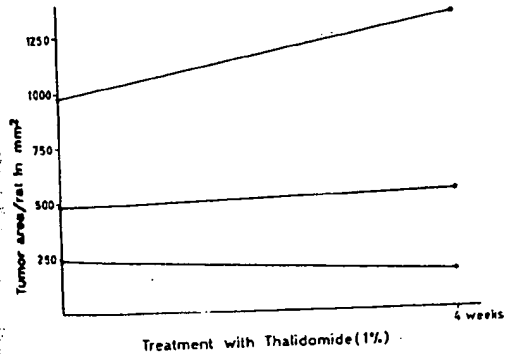


Fig. 5. *Change in size after 1% thalidomide treatment of tumors having areas of 200, 500, and 1,000 mm² before treatment.*

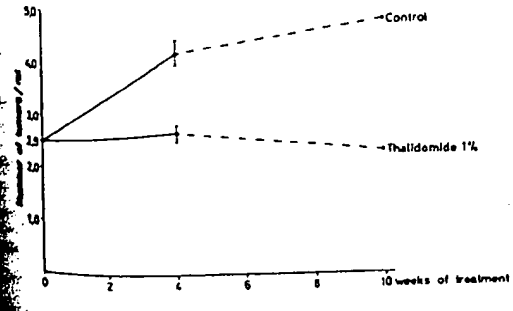


Fig. 6. *Appearance of new tumors during treatment in 10 curative experiments.*

of experiments, it was not possible to calculate the significance in this instance.

To elucidate the question of whether the curative influence on the DMBA tumors was a specific effect of thalidomide or was due to its sedative action, comparative trials were made with the following sedatives, glutethimide (0.4%), phenobarbital (0.16%), reserpine (0.001%), and chlorpromazine (0.2%).

Figure 7 shows the behaviour of the DMBA tumors in regard to these neuropharmaca compared with 1% thalidomide and with the control tumor animals. The mean tumor areas ranged from

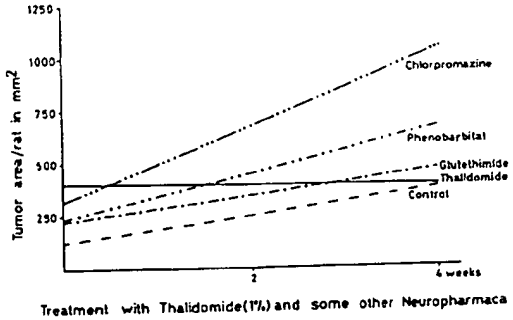


Fig. 7. *Treatment of DMBA-induced tumors with thalidomide in comparison with other neuropharmaca.*

about 200 to 400 mm² at the start of the 4-week experiment. In the animals given glutethimide, phenobarbital, or chlorpromazine, there was no significant difference from tumor development in the controls, whereas thalidomide under the same experimental conditions produced a reduction of the mean area of the tumors.

In a later experiment in which the animals had a mean tumor area of about 500 mm² at the beginning, thalidomide, glutethimide, reserpine, and phenobarbital had a definite influence on the development of the tumors, but no effect could be found with chlorpromazine. For reasons so far not explained, the animals' weight in this experiment, as opposed to that previously described, fell markedly during the treatment.

After about 6 weeks of treatment, the action of thalidomide, glutethimide, reserpine, and phenobarbital definitely abated.

The rats on the diet of 1% thalidomide pellets consumed less food during the experiment than did the controls. To clarify the question of how far the limitation of food intake caused by the thalidomide content influenced the antitumor action of the drug, "pair-feeding" experiments were made in which control tumor rats received the same amount of food as was eaten by their thalidomide-treated counterpart.

Figure 8 shows that in the thalidomide-treated rats there was a definitely slower growth rate of the tumors as compared with those of the controls. At the same time, the average body weight of the thalidomide rates was definitely increased (+19.8 g per animal) as opposed to the controls (-1.4 g per animal).

According to Huggins (1965), DMBA tumors in SD rats can be favorably influenced by the combined use of estradiol (0.02 mg) and progesterone (4.0 mg). Figure 9 shows the effect of this combination of hormones when the tumors have an average area of 300 to 500 mm² or else 800 mm² at the beginning of the experiment. In both experiments, the action of the hormone combination closely paralleled that of thalidomide, namely, stationary behavior of the tumor at the starting level of about 300 to 500 mm² mean tumor area per rat, and a progressive growth when the starting level was about 800 mm².

On the other hand, cyclophosphamide has only a limited action on manifest DMBA tumors, as can be seen in Fig. 10. Independent of the initial size of the tumor, under cyclophosphamide treatment the areas grew steadily, but still at a definitely slower rate than in the controls.

Altogether, the curative experiments carried out so far enable us to conclude that a 1% concentration of thalidomide over a period of 4 to 5 weeks limits the growth of DMBA-induced tumors, and in general this effect is more pronounced the smaller the tumors are at

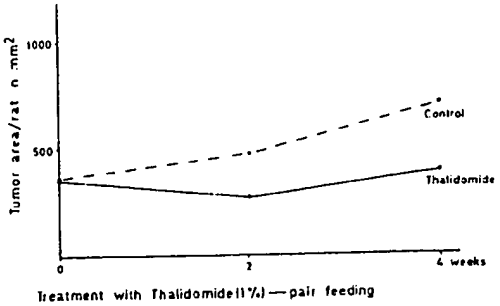


Fig. 8. Pair-feeding of 1% thalidomide-treated rats and untreated control animals.

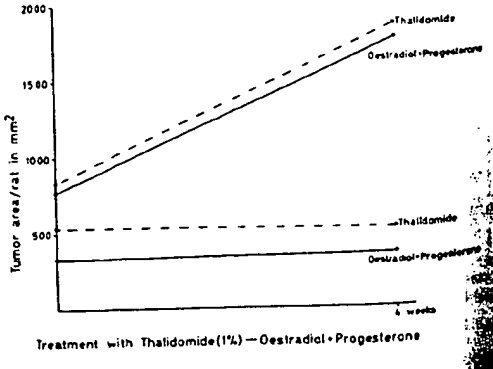
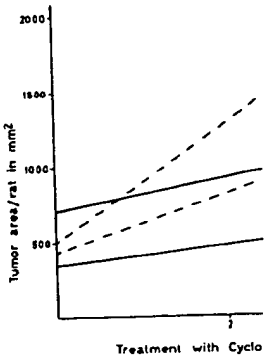


Fig. 9. Effect of estradiol and progesterone on size of tumors in rats receiving 1% thalidomide.



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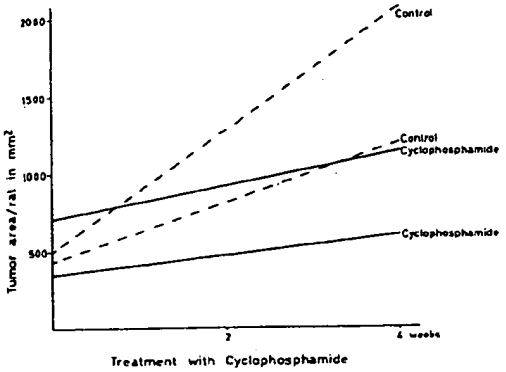

Fig. 10. *Effect of cyclophosphamide on the size of tumors in rats receiving 1% thalidomide.*

the start of treatment. With increasing tumor size, i.e., with greater age of the lesion, thalidomide effectiveness appears to diminish. If the treatment is prolonged beyond 5 weeks, the action of the drug seems to be reduced, and when it is stopped the tumors grow again at rates comparable with those of the controls.

Over and above this, thalidomide exerts a continuing effect of reducing the appearance of new tumors during the course of treatment. This effect seems to be largely independent of the size of the tumors at the start of the experiments or of the duration of treatment. As with the prophylaxis experiment, the curative action of thalidomide also resembles the effect of combined estradiol-progesterone treatment.

The neuropharmaca investigated behave differently towards DMBA tumors. Whereas in all the tests chlorpromazine promotes rather than inhibits the growth of the tumors, glutethimide, phenobarbital, and reserpine showed no kind of antitumor activity on one occasion, and on another there was marked inhibition of tumor growth with definite loss of weight and interruption of the sexual cycle. This will be reported more fully elsewhere.

Cyclophosphamide cannot hinder the growth of DMBA-induced tumors, even if their development is slower than in the untreated controls.

Obviously, the food intake has special significance. Limitation of the dietary quota, whether it be from reduced amounts given by the investigators, or from rejection by the animals, has a lasting effect on the behavior of the tumors. In evaluating the possible antitumor action of any substance, this effect must unquestionably be considered. The pair-feeding trial showed, however, that the thalidomide action did not depend on food restriction. According to Huggins (1965), the DMBA-induced tumors are generally hormone-dependent for a limited time, during which they may be favorably influenced by interference with the endocrine system, i.e., by deprival of hormones through hypophysectomy, ovariectomy, adrenalectomy, or by administering hormones. Thus, one comes to the conclusion that thalidomide primarily exercises its antitumor action via the endocrine system. This will be discussed in detail elsewhere.

Histopathological investigations, although not reported in detail in this paper, disclosed interesting histological changes in the tumor cells during thalidomide treatment, in the sense of a reversal of the dedifferentiation and increased maturity. These observations will also be described in a subsequent communication.

Spontaneous Tumors

To study spontaneous tumors, we used the virus (milk-factor)-determined, hormone-independent mammary carcinoma of C_3H/O_{20} mice. In contradistinction to the SD rats, which mostly succumb irregularly and only after a long time, these affected mice died relatively quickly and as a rule from the mammary carcinoma. After some pilot experiments, we chose the survival time of the mice, as well as the number and size of the tumors, as the most important criteria for estimating the chemotherapeutic effect.

As Fig. 11 and 12 show, under the conditions of the experiments described above, thalidomide did not influence in

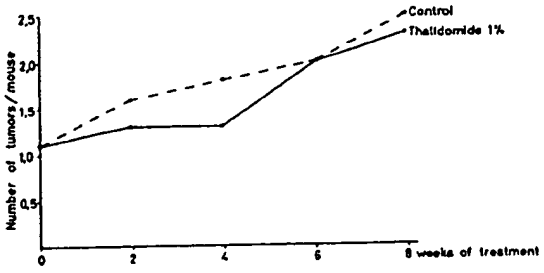

Fig. 11. *Effect of 1% thalidomide on the number of spontaneous tumors in mice.*

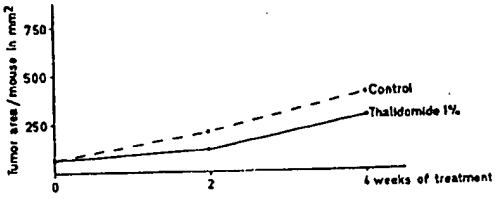

Fig. 12. *Effect of 1% thalidomide on the size of spontaneous tumors in mice.*

a lasting way either the number or the growth of tumors in the C_3H/O_{20} mice. At the start, there was a perceptible reduction both in the manifestation and growth of the tumors, but this effect weakened after a few weeks so that the difference from the untreated controls became smaller and smaller. Also, the survival time was not significantly influenced by 1% thalidomide.

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CLINICAL PHARMACOLOGY and THERAPEUTICS

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Clinical experiences with thalidomide in patients with cancer

Thalidomide (N-phthaloylglutamimide) was used as a screening agent in the treatment of 71 patients with a wide spectrum of cancers. The production of embryonic defects by this compound prompted us to use it as an anticancer agent. There was no evidence of objective response in any except one patient with renal cell cancer whose pulmonary lesions disappeared after treatment. This patient had also had nephrectomy, and it is highly possible that this response represented spontaneous regression of pulmonary metastases after operation, an event previously observed with this tumor.

Harry Grabstald, M.D., and Robert Golbey, M.D. *New York, N. Y.*
Urology Service, Department of Surgery and Chemotherapy Service, Department of Medicine, Memorial and James Ewing Hospitals; and the Sloan-Kettering Institute for Cancer Research

Thalidomide (N-(2,6-dioxo-3-piperidyl)-phthalimide) is an effective hypnotic drug,[9, 12, 13] and its untoward activity in producing abnormalities in the development of the human fetus is now well known.[7, 9]

As a hypnotic, prior to the discovery of its relationship to congenital anomalies, it had relatively few toxic effects. It did not cause respiratory depression in animals, and even large doses did not produce anesthesia, narcosis, or death. Attempts at suicide by the use of large doses of thalidomide were unsuccessful.[1, 11] The major manifestation of thalidomide toxicity in patients taking it for its hypnotic effect has been the infrequent appearance of a peripheral polyneuritis,[2] usually in the upper extremities; this resolved in most cases after the drug was stopped, but it sometimes persisted for many months.

Several drugs currently used for their antitumor activity are also teratogenic in animals.[10] The highly specific action of thalidomide in causing developmental defects in the fetuses of mothers ingesting this material during the critical period of gestation (fourth to seventh week) suggested the possibility that certain cancers might be adversely affected by the drug. An empirical trial of the drug was necessary, since the mechanism of action of thalidomide on the human fetus has not been elucidated, and the cellular system sensitive to its action is not known.

Materials and methods

Clinical material. In three separate institutions a total of 71 patients with advanced metastatic cancer was treated with thalidomide. Series A consisted of 30 pa-

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The drug used in our study was supplied by The Wm. S. Merrell Co., Division of Richardson-Merrell, Inc., Cincinnati, Ohio.

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Table I. *Distribution by diagnosis of patients treated with thalidomide*

Diagnosis	Series A	Series B	Series C	Total
Kidney	6	1	0	7
Bladder	5	0	0	5
Testes				
Choriocarcinoma	1	0	0	1
Embryonal carcinoma	2	0	0	2
Teratocarcinoma	3	0	0	3
Prostate	2	0	0	2
Gynecologic cancers	2	3	7	12
Breast	1	0	3	4
Digestive tract	1	4	6	11
Parotid	0	1	1	2
Lung	4	3	4	11
Lymphoma	0	0	4	4
Multiple myeloma	1	0	0	1
Melanoma	0	1	0	1
Thyroid	0	0	1	1
Gingiva	0	0	1	1
Sarcomas	2	0	1	3
Total	30	13	28	71

tients treated at Memorial Hospital; Series B comprised 13 patients treated by Dr. Stanley N. Levick at Moss Rehabilitation Hospital in Philadelphia, Pa.; and there were 28 patients in Series C, treated by Dr. A. Hochman at the Hadassah University Hospital, Jerusalem, Israel. The clinical diagnoses are shown in Table I.

Dosage. There was great variability in the total doses of thalidomide received by each patient, and some variation in the dosage schedules set up at the three institutions. In Series A an attempt was made to give 2 Gm. daily but frequently this was not feasible because of drowsiness. The patients who tolerated this total dose received 400 mg. three times during the day and 800 mg. at the hour of sleep. When, however, drowsiness prevented such simple self-care activities as eating, shaving, and bathing, the daily dosage was reduced. The patients were informed of the nature of the drug and the possibility that it would have a hypnotic action. Some of the subjects who were being treated as outpatients would decrease their drug intake voluntarily when they became excessively drowsy. The total dosage administered in this series ranged between 2 and 112 Gm. In Series B each patient received 400 mg. four times daily—on awakening, at 2:00 P.M., at 7:00 P.M., and at bedtime. The range of the total dose was from 1.2 Gm. to 132 Gm., with 10 of the patients receiving between 20 to 80 Gm. total dose. The dose range was somewhat smaller in Series C. The daily dose was between 300 and 900 mg. daily over a period of 3 to 6 weeks, and the total dose ranged between 18 and 37.8 Gm.

It is not known whether much larger doses might be capable of producing some evidence of anticancer activity. The limiting toxicity in determining the dose of the drug tolerated by the patient was disabling lethargy.

Methods of observation. The patients were treated in the hospital and in the outpatient department. The outpatients were examined at least once each week and had complete blood counts, urinalyses, and liver and kidney function tests at appropriate intervals; additional observations relating to the nature and location of the cancer were regularly made in order to demonstrate evidence of therapeutic activity.

Results

Toxic manifestations of thalidomide.

Rash. A generalized maculopapular rash associated with pruritus developed in 8 patients. In one patient the rash cleared when the drug was discontinued, but recurred when the drug was restarted. The rash then disappeared while the drug was continued and did not reappear again. Peripheral neuritis subsequently developed. In another subject a rash appeared one week before the patient died and was present at the time of death. The rash cleared in all the other patients on discontinuing thalidomide.

Thrombophlebitis. Four patients showed typical signs and symptoms of thrombophlebitis of the legs, which subsided when the drug was discontinued.

Polyneuritis. Two patients developed

evidence of peripheral polyneuritis, one characterized by numbness and tingling of the fingers and one by numbness of the tongue and lips. In both patients symptoms cleared when the drug was discontinued.

Nausea and vomiting. This was mild and was observed in only one case.

Amenorrhea. In one patient, with a diagnosis of metastatic melanoma, amenorrhea developed one month after starting thalidomide. She had previously had a temporary delay in the menses while being treated with actinomycin D, methotrexate, and Leukeran. This effect of thalidomide had previously been reported by Howe.[3]

Thalidomide did not have an adverse

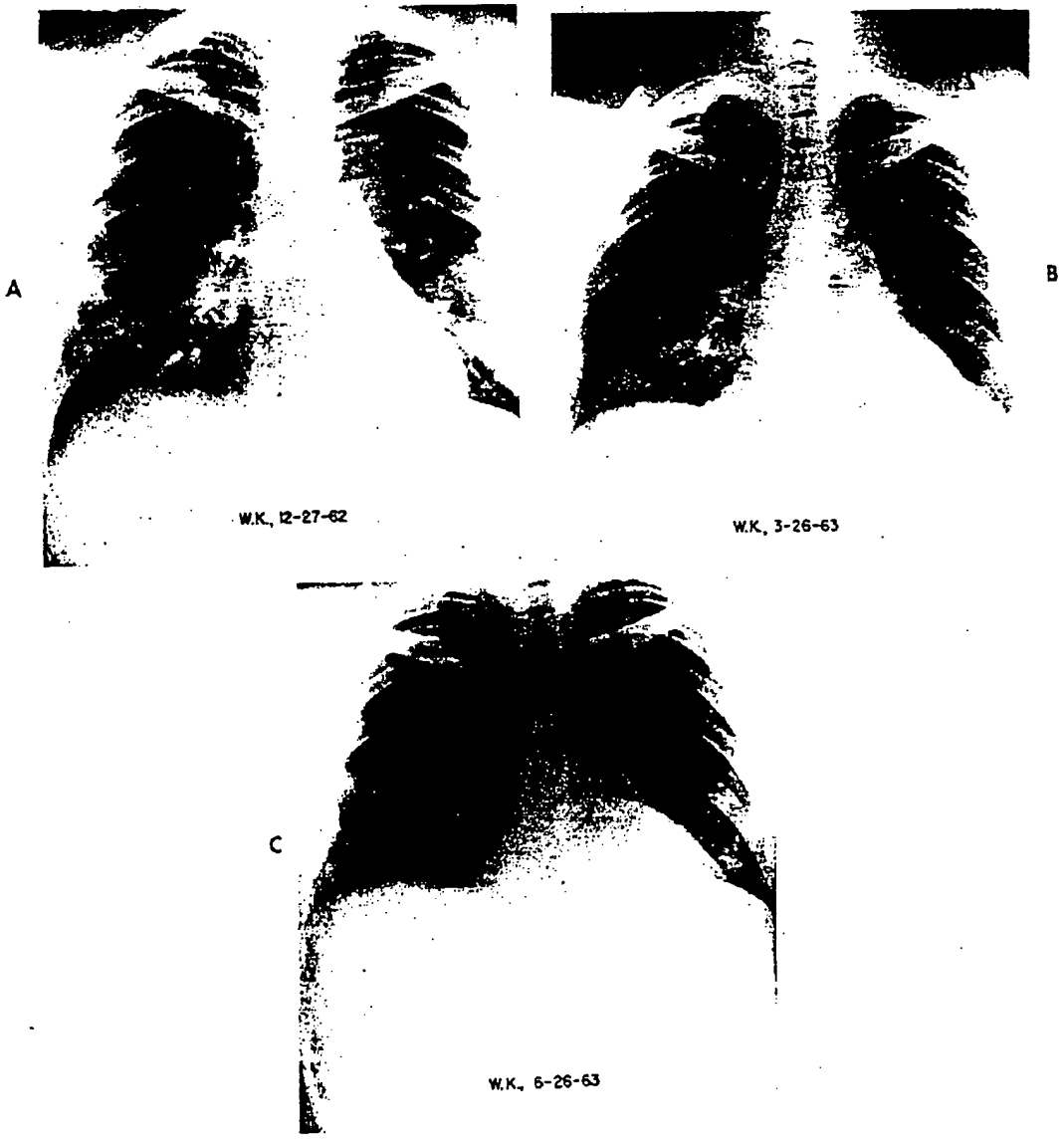

Fig. 1. Roentgenograms of the chest demonstrating the regression of pulmonary metastases occurring after nephrectomy for renal cell carcinoma, and at 3 and 6 months on thalidomide therapy.

effect on the peripheral blood picture. There was no evidence of kidney or liver toxicity attributable to thalidomide. In those cases in which azotemia was present, there was evidence of hydronephrosis and/or other renal disease to account for the findings. When liver function studies were abnormal this could be explained by the presence of hepatic metastases.

Therapeutic activity on cancer. No definite clinical responses were observed in the patients treated in Series B.[3] One patient with endometrial carcinoma did not show any apparent progression of the disease for several months during treatment. There was, however, no decrease in the size of abdominal masses, and after several months the growth of the cancer became apparent and the patient died one year after the onset of therapy. This course is not incompatible with the natural evolution of untreated disease. One case of endometrial carcinoma in each of the other two series showed no effect.

In Series C no objective improvement was observed in any of the 28 patients.[4]

In Series A only 1 of 30 patients demonstrated objective benefit conceivably related to the administration of thalidomide. This case is of interest and is presented in detail.

The patient was a 42-year-old male who had a nephrectomy for renal cell carcinoma in November, 1962. Prior to the operation, a chest x-ray showed bilateral pulmonary metastases and, at operation, a lymph node found at the bifurcation of the aorta was histologically positive for metastases. Postoperatively the pulmonary metastases appeared to be enlarging by x-ray examination and the patient was referred to Memorial Hospital for further treatment. Twenty-five days after operation the subject was started on thalidomide, 2 Gm. per day. This dose produced profound lethargy and so was decreased to a tolerated level of a single dose of 200 mg. in the morning and 800 mg. at bedtime. After 2 weeks on the drug a diffuse punctate rash associated with pruritus and fever developed. The drug was discontinued and the rash improved over a 4 day period. The rash had disappeared within 2 weeks; thalidomide was restarted at 1 Gm. per day. The rash recurred and slowly subsided spontaneously while therapy continued. The drug was otherwise well tolerated and the dose was increased to 600 mg. in divided doses through the day, and 800 mg. at bedtime. A chest film taken 3 months after the onset of therapy showed almost complete disappearance of the pulmonary metastases (Fig. 1). Three months later there was no definite evidence of residual tumor. In May, 1963, 5 months after the onset of thalidomide administration, the patient had gained 30 pounds in weight, from 170 to 200 pounds. Two months later, in July, 1963, the subject first noted numbness and tingling of the fingers. A neurological consultation confirmed the diagnosis of peripheral neuritis secondary to medication, and the drug was discontinued. One month later the patient had a Jacksonian seizure secondary to brain metastases. Despite transient relief of symptoms with symptomatic medication and radiation therapy to the brain, the patient had relatively steady progression of the neurological symptoms until his death in December, 1963.

The pulmonary metastases that were first noted to have disappeared in late March, 1963, did not begin to increase in size and number until October, 1963, and then they grew rapidly during the last month before death.

Several cases of renal cell carcinoma have been reported in which spontaneous regression of pulmonary metastases was observed after nephrectomy; this case could represent another such instance.[3] Six other patients with renal cell carcinoma treated with thalidomide showed no evidence of benefit.

Discussion

These three independent series comprising 71 patients represent a clinical trial of thalidomide against a wide spectrum of human cancers. No significant degreee of antineoplastic activity was demonstrated, although the possibility that a particular type of cancer may show some degree of response if a larger series of patients were treated has not been ruled out. In the absence of more definite evidence of pharmacologic or anticancer effects in man, we conclude that further random trials of this drug against cancer in man are not indicated. If laboratory studies subsequently indicate that thalidomide acts on specific biochemical processes, more extensive trials may then be indicated in forms of cancer with biochemical pathways relevant to the

known mechanisms of action of thalidomide.

The experience with thalidomide indicates that it is a useful hypnotic, and may be used in males and in females in whom there is no possibility of pregnancy. The major toxic manifestations seen were peripheral neuritis and transient skin eruption—both of which subsided when the drug was discontinued—and, possibly, and for unexplained reasons, thrombophlebitis.

Conclusions

Thalidomide was studied as an anticancer drug in a series of 71 patients with a variety of cancer, from three different institutions. The production of embryonic defects in the human fetus by thalidomide prompted its trial as an anticancer agent. No evidence of objective regressions was obtained, with the exception of one patient with renal cell cancer whose pulmonary metastases disappeared transiently after treatment. Since this patient also had a nephrectomy preceding the regression, the response may be attributed to this operation.

We wish to express our thanks to the following individuals and institutions for their cooperation and permission to include data concerning their patients in this report: Drs. Stanley N. Levick, Irving Waldo, and Yeng Wen-Huang of the Moss Rehabilitation Hospital, Philadelphia, Pa.; and Dr. A. Hochman, Director, Department of Oncology, Hadassah University Hospital, Jerusalem, Israel.

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